

What is claimed is:

1 A fusion protein comprising:

- 2 *Sub*
3 *cl* a) a reporter polypeptide linked to a linker polypeptide comprising a protease
4 cleavage site; and
5 b) a repressor polypeptide that represses the activity of said reporter polypeptide,
6 wherein said repressor polypeptide is operatively linked to the linker polypeptide,
7 and
8 wherein cleavage of said linker polypeptide at said protease cleavage site increases the
activity of said reporter.

1 2. The fusion protein of claim 1, wherein said protease cleavage site is a caspase cleavage
2 site.

3 3. The fusion protein of claim 1, wherein said repressor polypeptide comprises a nuclear
4 export sequence that directs the localization of said fusion protein outside of the nucleus
5 of a cell.

6 4. The fusion protein of claim 3, wherein said repressor polypeptide is an N-terminal
7 fragment of CD4.

1 5. The fusion protein of claim 3, wherein said reporter polypeptide is a transcription factor.

1 6. The fusion protein of claim 5, wherein said transcription factor is C-terminal LexA-B42
2 transcription factor.

1 7. The fusion protein of claim 3, wherein said repressor polypeptide is amyloid precursor
2 protein.

1 8. The fusion protein of claim 1, wherein said reporter polypeptide is a kinase.

- 1 9. The fusion protein of claim 1, wherein said reporter polypeptide and said repressor
2 polypeptide are fluorescent polypeptides, and wherein fluorescence energy transfer
3 occurs between said reporter polypeptide and said repressor polypeptide, wherein
4 cleavage at said protease cleavage sites results in an alteration in fluorescence energy
5 transfer between said reporter polypeptide and said repressor polypeptide.
- 1 10. A nucleic acid encoding the fusion polypeptide of claim 1.
- 1 11. A host cell comprising the nucleic acid of claim 10.
- 1 12. The host cell of claim 11, wherein said cell is a prokaryotic cell.
13. The host cell of claim 11, wherein said cell is a eukaryotic cell.
14. The host cell of claim 13, wherein said cell is a yeast cell.
15. The host cell of claim 13, wherein said cell is a human cell.
16. A method for identifying a protease that recognizes a specific protease cleavage site,
comprising:
- a) providing cell comprising a fusion protein comprising a reporter polypeptide
linked to a linker polypeptide comprising a protease cleavage site; and a repressor
polypeptide that represses the activity of said reporter polypeptide, wherein said
repressor polypeptide is operatively linked to the linker polypeptide, and wherein
cleavage of said linker polypeptide at said protease cleavage site increases the
activity of said reporter polypeptide;
 - b) expressing a test protease in said cell; and
 - c) comparing the activity of said reporter in said cell expressing said test protease
with expression of said reporter in a cell not expressing said test protease, wherein
and increased activity of said reporter indicates cleavage of the protease cleavage
site by said test protease.

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- 1 17. The method of claim 16, wherein said cell is a prokaryotic cell.
- 1 18. The method of claim 16, wherein said cell is a eukaryotic cell.
- 1 19. The method of claim 18, wherein said cell is a yeast cell.
- 1 20. The method of claim 19, wherein said cell is a mammalian cell.
- 1 21. The method of claim 19, wherein said cell is a human cell.
- 1 22. The method of claim 19, wherein said repressor polypeptide comprises a nuclear export sequence that directs the localization of said fusion protein outside of the nucleus of a cell.
23. The method of claim 22, wherein said repressor polypeptide is an N-terminal fragment of CD4.
24. The method of claim 22, wherein said repressor is amyloid precursor protein.
25. The method of claim 16, wherein said reporter is a transcription factor.
- 1 26. The fusion protein of claim 25, wherein said transcription factor is C-terminal LexA-B42 transcription factor.
- 1 27. The method of claim 25, wherein said cell further comprises a nucleic acid encoding a binding site for said transcription factor operatively linked to a nucleic acid encoding a second reporter.
- 1 28. The method of claim 27, wherein said second reporter is lacZ.

1 29. The method of claim 27, wherein said second reporter confers expression dependent-
2 toxicity.

1 30. The method of claim 29, wherein said second reporter is URA3.

1 31. The method of claim 16, wherein said protease cleavage site is a caspase cleavage site.

1 32. The method of claim 16, wherein the protease cleavage polypeptide is a protease
2 cleavage site in the amyloid precursor protein.

1 33. A method of identifying a compound that inhibits a protease, comprising:

a) providing a cell comprising

i) a fusion protein comprising a reporter polypeptide linked to a linker polypeptide comprising a protease cleavage site; and a repressor polypeptide that represses the activity of said reporter, wherein said repressor polypeptide is operatively linked to the linker polypeptide, and, wherein cleavage of said linker polypeptide at said protease cleavage site increases the activity of said reporter polypeptide, and

ii) a protease that cleaves at said protease cleavage site;

b) contacting said cell with said compound under conditions sufficient for said components to interact; and

c) measuring the activity of said reporter, wherein a decrease in the activity of the reporter indicates an ability of the compound to inhibit the protease.

1 34. The method of claim 33, wherein said cell is a prokaryotic cell.

1 35. The method of claim 33, wherein said cell is a eukaryotic cell.

1 36. The method of claim 35, wherein said cell is a yeast cell.

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- 1 37. The method of claim 35, wherein said cell is a mammalian cell.
- 1 38. The method of claim 37, wherein said cell is a human cell.
- 1 39. The method of claim 33, wherein said reporter is a transcription factor.
- 1 40. The method of claim 39, wherein said wherein said cell further comprises a nucleic acid
2 encoding a binding site for said transcription factor operatively linked to a nucleic acid
3 encoding a second reporter.
- 1 41. The method of claim 40, wherein said second reporter confers expression dependent-
2 toxicity.
42. The method of claim 41, wherein said second reporter is URA3.
43. The method of claim 33, wherein said protease is caspase, and said protease cleavage site
is a caspase cleavage site.
44. The method of claim 33, wherein said repressor is amyloid precursor protein.
45. The method of claim 33, wherein said protease cleavage site is a amyloid precursor
protein protease cleavage site.

- 1 46. A method of identifying a compound that activates a protease, comprising:
2 a) providing a cell comprising
3 i) a fusion protein comprising a reporter polypeptide linked to a linker
4 polypeptide comprising a protease cleavage site; and a repressor
5 polypeptide that represses the activity of said reporter, wherein said
6 repressor polypeptide is operatively linked to the linker polypeptide, and,
7 wherein cleavage of said linker polypeptide at said protease cleavage site
8 increases the activity of said reporter polypeptide, and
9 ii) a protease that cleaves at said protease cleavage site;
10 b) contacting said cell with said compound under conditions sufficient for said
11 components to interact; and
12 c) measuring the activity of said reporter, wherein an increase in the activity of the
reporter indicates an ability of the compound to activate the protease.

47. The method of claim 46, wherein said cell is a prokaryotic cell.

48. The method of claim 46, wherein said cell is a eukaryotic cell.

49. The method of claim 48, wherein said cell is a yeast cell.

50. The method of claim 48, wherein said cell is a mammalian cell.

51. The method of claim 50, wherein said cell is a human cell.

52. The method of claim 46, wherein said reporter is a transcription factor.

53. The method of claim 52, wherein said wherein said cell further comprises a nucleic acid
encoding a binding site for said transcription factor operatively linked to a nucleic acid
encoding a second reporter.

1 54. The method of claim 46, wherein said protease is caspase, and said protease cleavage site
2 is a caspase cleavage site.

1 55. The method of claim 46, wherein said repressor is amyloid precursor protein.

1 56. The method of claim 46, wherein said protease cleavage site is a amyloid precursor
2 protein protease cleavage site.

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